Chapter

Honey Production Process

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Abstract

Honey has been considered as a very important and superior nutrient in human nutrition since ancient times due to its ability to be consumed by humans without processing, easy digestibility, nutritional properties and biological benefits. Although honey contains many desired bioactive and antibacterial substances, which may be sufficient for antimicrobial activity, it cannot be produced in sufficient quantities due to low water activity under normal conditions. This causes various food and bee-borne spores/non-spores pathogens going viral. Hence, it may cause the risk of parasitological and fungal agents to be found. In honey production, "Hazard Analysis Critical Control Point (HACCP)" must be applied meticulously and completely. Current technologies in honey production will be explained in this section.

Keywords: honey production, microbiological risks, HACCP

1. Introduction

Honey consumption has a very old history for humans. It has been used as a sweetener and flavoring in countless food and beverages. Since ancient times, honey has been known for its nutritious and therapeutic aspects. The most important components of honey are carbohydrates, which are found in the form of fructose, glucose, disaccharides, and oligosaccharides, and components such as maltose, isomaltose, maltulose, sucrose provide the sweet taste to honey. It also contains enzymes such as amylase, oxidase peroxide, catalase and acid phosphorylase, including anderosis and panoz. Also, honey is rich in amino acids, minerals, antioxidants and various phytochemicals [1]. Many of the reported biological properties of honey, such as antioxidant, antibacterial, antifungicidal, anti-inflammatory, hypotensive, antiproliferative, hepato-protective properties of these components are associated with presence of these properties. However, the composition of honey largely depends on a number of factors, such as flower source, geographical region, climatic conditions, harvesting season, processing and storage conditions. There are studies that report that honey, administered alone or in combination with traditional therapy, may be useful in the treatment of chronic diseases that are commonly associated with oxidative stress and the state of inflammation [2]. Honey is classified according to various criteria. In this classification, honey is classified as secretion honey (such as pine honey, oak honey, fir honey, leaf honey) and flower honey (linden honey, cotton honey, trirose honey, thyme honey, mashed honey, acacia honey, heather honey, etc.). According to the form of marketing, framed comb honey, natural comb honey, partial comb honey, cut-comb honey, strained honey, crystallized honey, creamed honey, pressed honey, chunk honey (strained with comb or comb with strained), filtered honey and baker's honey. According to the moisture content, honey is classified as grade 1 honeys (humidity below 17.8%), grade 2 honeys (humidity up to 18.6%) and grade 3 honeys (humidity up to 20%). According to their color, honey is classified as white, golden, amber and dark. The color of honey can vary from light water white to black warehouse [3]. The physical and chemical properties, antimicrobial effects, which are of great importance for public health, and GMP and HACCP systems applied in the production process and microbiological dangers will be addressed in this section.

1.1 Physical and chemical properties of honey

Honey contains about 200 substances and is a nutrient consisting of substances such as carbohydrates, water, enzymes, free amino acids, essential minerals, vitamins, phenolic compounds, volatile compounds (monoterpenes, benzene derivatives) and some other solids. Carbohydrates in honey are mainly monosaccharides, glucose and fructose. This is followed by disaccharides and trisaccharides. They contribute mainly to the energy value. Proteins include enzymes such as invertase, diastase, glucose oxidase, catalase, peroxidase and acid phosphatase, and their content varies from 0.1% to 3.3% depending on the type of honey. It contains essential and non-essential amino acids, but the most common amino acid in honey is proline, which accounts for 1% of honey components [2]. Honey contains tocopherol (E), anti-hemorrhagic vitamin (K), ascorbic acid (C), thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5) and a small amount of vitamin pyridoxine (B_6). Vitamins of the B complex and vitamin C are mainly derived from pollen and can be affected by commercial and industrial processes such as filtration or oxidation reactions [4].

Honey has a slight acid reaction due to about 0.57% organic acids. Acids contribute to the aroma and antimicrobial activity of honey. The predominant acid in honey is gluconic acid, it is followed by aspartic citric, acetic, formic, fumaric, galacturonic, malonic, formic, acetoglutaric, glutamic, butyric, glutaric, propionic, pyruvic, glioxia, 2-hydroxybutyric, a-hydroxiglutaric, isocyric, lactic, malic, methylmalonic, kynic, succinic, tartaric, oxalic acid [2]. The mineral content in honey ranges from 0.04% in light honey and 0.2% in dark honey. Potassium is the most abundant element. But the main bioactive molecules contained in honey are represented by polyphenols. Polyphenols are a heterogeneous chemical compound that can be divided into flavonoids (flavonols, flavones, flavanols, flavanones, anthocyanin, calcones and isoflavones) and non-flavonoid (phenolic acids). The profile of polyphenolic compounds in honey is thoroughly studied and includes vanillin, caffeic, syringing, p-gamic, ferulic, ellagic, 3-hydroxybenzoic, chlorogenic, genistic, gallic and benzoic acids and contains different phenolic acids, such as different flavonoids, mainly quercetin, kaempferol, myricetin, chrysin, galangin, hesperetin. The amount and type of polyphenols largely depends on the flower source or the variety of honey. In addition, it is known that there is a strong relationship between antioxidant activity and total phenolic content [5].

1.2 The importance of honey in terms of health and its antimicrobial effect

Honey is a food that has been used in therapeutic treatments for thousands of years. Among other useful properties to health, this product has been reported as a promising agent for wound healing, including leg ulcers and eyes, skin disorders by in vitro and clinical studies. In studies on New Zealand manuka honey, unique to the New Zealand, positive effects were observed on the viability of potentially useful *Lactobacillus reuter* and *Bifidobacterium longum* found in the human intestine. Moreover, it was found that *Salmonella enterica* Typhimurium, an enteric pathogenic bacterial type, showed a 65% reduction in their proliferation. In this sense, it has been established that manuka honey has a beneficial effect on the intestine by producing acid metabolites that reduce the intestinal pH and prevent pathogenic colonization and hence support the growth of bifidobacteria and lactic acid bacteria. Honey has been reportedly able to modulate oxidative stress and also has anti-proliferative, pro-apoptotic, anti-inflammatory and anti-metastatic properties. The anticancer effect of honey is connected to the presence of natural bioactive compounds, mainly such as pinobanksin, pinocembrin, luteolin, chrysin, salicylic acid and 3.4 dihydroxybenzoic acid [6, 7]. Some of the vitamins contained in honey are ascorbic acid, pantothenic acid, niacin and riboflavin. Moreover, it is a food that also contains minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc. Its rich variety of vitamins and minerals also plays a role in increasing the antioxidant characteristics of honey. The presence of free radicals and reactive oxygen types is responsible for pathogenesis of aging, as well as cellular dysfunction, metabolic and cardiovascular diseases. Consumption of foods rich in antioxidants can protect against these pathological changes, preventing the pathogenesis of chronic ailments [8].

Various parameters such as low water activity, high sugar content, acidity and hydrogen peroxide (H_2O_2) content, phytochemicals, peptides, non-peroxidase glycoeptides and proteins make up the antibacterial potential of honey. Water activity of honey varies from 0.56–0.62. These values might be considered low enough to prevent the development of bacteria or other microorganisms [9]. Although previously it was believed that the only responsible agent for the antibacterial effect of diluted honey was H_2O_2 and that this antibacterial effect can be completely eliminated through catalysis, it has been found out that bacteria can also be affected via the existence of pythochemical elements present in honey [10]. As it suppresses the activities of bacteria causing infections in urinary systems, such as *E. coli* and *Proteus* species and *Streptococcus faecalis*, diluted honey is used to treat urinary system infections and it inhibits toxin production [11]. Undiluted honey hinders the reproduction and development of bacteria due to the content of sugar, which exerts osmotic pressure on bacterial cells and causes water to flow out of bacterial cells through osmosis. Thus, the cells shrink due to dehydration, and they cannot remain alive in hypertonic sugar solution. The optimal pH necessary for the development of most microorganisms ranges from 6.5–7.5. The pH value of honey is between 3.2–4.5, and this value is a very distinctive feature of its antibacterial activity. This acidity is caused by the presence of certain important organic acids, especially gluconic acid - in 0.5% (a/h) concentration. Glycogenic acid is produced from glucose oxidation by an endogenous enzyme of glucose oxidase and is an extremely powerful antibacterial agent. In undiluted pure honey, low pH can contribute to antibacterial action, but when the product is diluted pH alone is not enough to prevent the development of bacteria [9]. The formation of H_2O_2 is a dominant mechanism in which honey exerts bacteriostatic and bactericidal activity. It provides antibacterial activity of honey and is produced enzymatically. The enzyme glucose oxidase is inherently inactive in honey due to low pH conditions, and glucose oxidase is activated when honey is diluted. However, it is known that concentrations of H_2O_2 are adversely affected by various minor components, such as nectar, pollen, and yeast. It has also been reported as having high sensitivity to light and light sources [12]. Honey contains relatively small amounts of proteins, whose molecular weights range from 20 to 80 kDa, ranging from approximately 0.1% to 0.5%. These proteins contain many enzymes involved in sugar metabolism, such as alpha and beta glucosidase, glucose oxidase and amylase. Numerous studies have shown that important royal jelly proteins have antimicrobial and anticancer activity and anti-inflammatory potential [13].

Honey shows antibacterial activity against a large number of bacteria in different environments. Natural components of honey have antifungal, antiviral, antibacterial activities. It has been reported that the antibacterial activity of honey is also likely to depend on the pasture, climatic conditions, and also on the natural composition of flower nectar. Honey has excellent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), often associated with wound and burn infections, and *Pseudomonas* spp. Many studies have shown that honey is also effective against hemolytic streptococci and vancomycin resistant enterococci. Twenty-one kinds of honey tested for antibacterial activity against *Staphylococcus aureus (S. aureus*) and *Pseudomonas aeruginosa (P. aeruginosa*), and it has been established that they have a positive effect due to H_2O_2 and polyphenolic content levels. The effectiveness of free radical cleansing is observed in all kinds of honey. In addition, honey tested by freezing, drying and powdering has been reported to show antioxidant activity in each form [9, 14]. Flavonoids contained in the natural composition of honey is also known to be effective against microorganisms that are present in the tissue of chronic wounds, in particular *S. aureus*, *P. aeruginosa*, as well as *Escherichia coli (E. coli*). Flavonoids are often recommended as a natural source to control chronic inflammatory diseases, the incidence of which increases significantly. Despite the fact that the topical application of honey for medicinal purposes is old, there are a small number of studies that address its anti-inflammatory activity at the cellular level. Although flavonoids are small components of honey, their anti-inflammatory effect is extraordinary compared to other natural compounds [15]. Honey was found to have a preventive effect on about 60 bacteria such as*, Bacillus anthracis, Corynebacterium diptheriae, Haemophilus influenzae, Klebsiella pneumoniae, Shigella, Mycobacterium tuberculosis*, and many aerob and anaerob bacterial types. In vitro studies of *Helicobacter pylori* in the human digestive system have shown that when using honey, its activity decreases by 20%. It has been reported that honey can be used in combination with antibiotics to produce a synergistic effect of bactericidal activity against *Helicobacter pylori*. The main difference of honey with antibiotics is that it does not develop antibiotic-resistant bacteria, so it can be used continuously without such risk [16].

2. Other beekeeping products

2.1 Pollen

Pollen is the only source of protein found in nature for bees. The amino acids contained in its composition are isolosin, arginine, lysine, histidine, leucine, methionine, treonine, phenylalanine, tryptophan and valine. It is essential for adequate development of their muscles, tissues, secretory glands and other organs in the upbringing of honeybees and its young stages. It is a nutritional source rich in vitamins, proteins, sterols, minerals and lipids. It has been reported that pollen collected by honeybees may have differences in their general chemical composition as a result of supplying from different plants [17].

2.2 Nectar

Bees have two stomach and they use one of them to perform normal body functions whereas the other to store the nectar they collect. In order to collect nectar found in flowers, bees use rod-like, tubular long tongues. It has been reported that bees can contain about 70 mg of nectar in the stomach they store nectar, and that they should visit 100 to 1500 different flowers to fully fill their honey stomach [17].

2.3 Propolis

Propolis is recognized as a therapeutic agent due to several reported functional effectiveness. It is known that honey contains phenolic compounds. Propolis contains a higher content of phenolic compounds than honey and shows significantly higher antimicrobial and antioxidant activities. Today it is used in industry as a component of confectionery, biopharmaceuticals and cosmetics. It is gaining popularity as a natural preservative and helps to improve shelf life and consumer health as a source of bioactive compounds for food and drinks. However, propolis has a strong and bitter taste, which changes the sensory properties of food due to the high concentration of phenolic compounds. Therefore, the acceptance of foods containing propolis by consumers must be determined by its propolis concentration, which has to be carefully researched so as not to adversely change the sensory properties of such foods [18].

2.4 Bee milk

The importance of bee milk, one of bee products, was noticed in the 1600s and was given the name "*Royal Jelly*", which means excellent food in English. Bee milk is secreted from the upper jaw (mandibular) and throat glands (hypopharyngeal) of young worker bees of 5–15 days of age. All larvae only in their first three-day period, and the larvae that will become the queen bee are fed with royal jelly during the entire larval and adult periods. Bee milk can be described as food with a peculiar smell and a bitter taste and a mush-like form with bone-like color. It is collected from the cells of larvae of 3–4 days of age of the future queen, or from the cells of queen bees where larvae of 1–2 days are laid after 48–72 hours. It is quite flowing and has yogurt-like consistency but is a homogeneous substance. It has a light beige and yellowish whitish color, a sharp phenolic smell and a distinctive sour taste. Its density is approximately 1.1 g/cm^3 and is soluble in water [19].

3. Microbiological risks in honey and honey products

Although honey is considered a low-risk food due to its antimicrobial and bacteriostatic effects, studies disprove this view. In addition to primary contamination, staff, tools and equipment used in beekeeping and honey production are also a potential source of secondary contamination. In addition, honey, which has the potential to contain many microorganisms as a result of cross-contamination, is among the important nutrients and can indirectly threaten public health. Despite the fact that some types of honey contain H_2O_2 and benzoic acid and phenolic compounds such as some flavonoids, it can constitute risks for consumer health due to minimal hygiene rules. It is reported that pathogens can be found as causative agents in honey produced without food safety systems. Food-borne pathogens are recognized as an important risk factor for public health in developed and developing countries due to their spread around the world. Viruses, bacteria, fungi, parasites and mites are the most common disease factors in beekeeping. Fecal-oral route is an important way of transmission of these diseases. Agents that pollute bees through water and food can be transmitted to larvae by infected bees. Another contamination that may occur in honey is secondary contamination caused by secondary contamination sources such as personnel, tools and equipment [20].

The presence of strains *Bettsya alvei*, *Acosphaera apis* and *Acosphaera* major in honey production can be indicative of improper beehive management practices. Different types of microorganisms such as *Acinetobacter* spp., *Bacillus* spp., *Clostridium* spp., *Corynebacterium* spp., *Pseudomonas* spp. are bacteria that are widely found in the soil. *Brochothrix* spp., *Citrobacter* spp., *Enterobacter* spp., *Erwinia* spp., *Flavobacterium* spp., *Lactobacillus* spp., *Lactococcus* spp., *Leuconostoc* spp., *Listeria* spp. and *Pediococcus* spp. are other bacteria that are likely to be found in plants and plant products. On the other hand, among yeast strains *Saccharomyces*, *Schizosaccharomyces* and *Torula* species predominate in high humidity sugars. Bacterial spores, especially *Bacillus* and *Clostridium*, can be seen in honey. *Clostridium* is an indicator organism that provides evidence of contamination or pollution in honey. *Clostridium botulinum* (*C. botulinum*) spores are usually found at low levels in honey. The presence of *clostridium* spores can be dangerous, especially for children under one year of age. It is known that infant botulism is mainly caused by the consumption of honey contaminated with *C. botulinum* [21, 22]. *C. botulinum* forms 4 different types of neuroparalytic diseases in humans. In addition to infant botulism, they are classified as food-borne botulism, wound botulism, and yet unclassified latent botulism. The most important of them is infant botulism, which occurs in newborn babies of 3–20 weeks. Infant botulism is diagnosed with isolation of *C. botulinum* and toxin in feces. Decreases in sucking and swallowing reflexes of infants can be observed, which is very rarely fatal [23].

One of the animal products that have been the focus of food warnings due to the presence of chemical hazards such as antibiotics or pesticides in recent years are honey and honey products. The source of these residues in honey is mainly due to bee parasites, such as European offspring rot (*Streptococcus pluton*) or American offspring rot (*Bacillus larvae*) and are veterinary drugs that are necessary to treat bacterial diseases. It is known that chemical residues caused by these drugs used to eliminate microbiological risks, lead to such adverse conditions on human health as allergic reactions, bacterial resistance, along with changes of reproductive toxicity [24, 25].

4. HACCP in honey production

Food safety can be ensured by systematic implementation of all activities in line with a plan. The Hazard Analysis Critical Control Points (HACCP) system, as a preventive system for ensuring food safety, controls production at various points throughout the food production, thereby ensuring that the final product complies with legislation. Preliminary Requirement Program must be created first in order to establish the HACCP system in any food business. In this context, the deficiencies of the infrastructure and processes such as water, energy, warehouse, cleaning and sanitation, personnel, environment and equipment hygiene, personnel training and pest control should be addressed. However, it is necessary to plan the process management by writing down the procedures. The processes that need to be addressed afterwards can be sorted as follows; identification of the HACCP team and a clear definition of the task descriptions by making the managerial organization chart, determination of food safety policy by business management, making an understandable description of the products to be produced, determination of the intended usage method, preparation of a flow diagram and placement plan by HACCP team and verification of this plan at site, analyzing hazards and risks, identification of critical control points, making up of critical limits and monitoring procedures, determination of corrective activities for cases where it is necessary, and the proving or verification of the effectiveness of the system [26].

Codex Alimentarius Standard and the European Commission allows nomenclature for honeys produced from certain botanical sources if the product comes from the specified origin and has anticipated physicochemical, organoleptic and microscopic properties. The fact that there are different varieties of honey and each has its own production steps, leads to an increase in the limits that need to be controlled. For the import of food products of animal origin, such as honey, EU legislation requires a number of health and national residue monitoring procedures such as HACCP during the production and processing of honey. These requirements are known to be independent of whether honey is organic or traditional. Thus, imported products are intended to meet the standards required for production and trade within EU member countries. Costs, lack of qualified personnel, misinterpretation of EU legislation, lack of laboratory in international standards and improper infrastructure are the main obstacles to being accredited by the EU [27, 28].

Although honey is a product that is part of the low-risk group due to its high sugar content, it should be carefully examined for physical, chemical and biological hazards. In general, the hygiene of the processing area, tool and equipment and personnel in contact with food should be observed as it should be in all food enterprises. Physical hazards such as soil, plant materials, glass materials, tools and equipment are defined as potential hazards to honey. Traces of pesticides and herbicide, beekeeping drugs and antibiotics are chemical hazards. Soil originated *C. botulinum*, the most important biological danger in honey production, is eliminated by the provision of hygienic conditions in the production of honey [29].

General hygiene rules should be applied effectively to prevent physical, chemical, and microbiological hazards. In hives, legally approved preservatives should be used. Insects and mice should be kept away from hives. During transportation, the vehicles should be cleaned well, in case of the presence of dirt left from the previous use. It is necessary to effectively clean the equipment and work area before and after use. Persons involved in the process should wear a separate clothing to protect the product from contamination caused by clothing or individuals. Especially before and after use, cleaning control of filters must be carried out effectively. In the HACCP plan, the purpose of conducting hazard analysis must be effectively controlled. All potential hazards in each step of the workflow process must be identified and the risk and severity of each identified hazard should be assessed. At this point, it is also necessary to determine the sources of dangers. In a study, corrective activities to prevent and/or eliminate hazards were determined and two critical controls points, “filtration/unloading” and “packaging” were pointed out. Examples of forms used in each HACCP plan and all procedures of the HACCP plan must be provided and monitored [26, 30].

5. Conclusions

HACCP system, which is successfully implemented in the food industry, is the most effective quality system in terms of the supply of safe products. The purpose of the use of HACCP system is to provide reliable food to the consumer with the desired characteristics and quality. Honey production, which is suitable to be affected by climatic conditions, should be made systematic and controllable by removing traditional methods that are difficult to trace. In this context, the creation of honey workflow process, determining potential hazards, and analyzing hazards, taking necessary precautions, recording the system, providing internationally reliable product guarantee is of great importance for public health as well as for the economies of countries. Effective implementation of the HACCP system in enterprises is inevitable so that retrospective monitoring and recall models can be used in the event of any negativity. In the production processes of foods with high nutritional value as honey, all necessary food safety requirements must be met to protect and improve public health.

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